REMARKS

Claim 1 has been withdrawn without prejudice. Claims 2, 3, 5, 6 and 9-11 are amended. Claims 2-11 are pending in this application.

Claim 2 has been amended to include subject matter from claim 1, which has been withdrawn without prejudice. Moreover, the term "viable" has been added before the word "cell" within the first clause of claim 2 and the phrase "by irradiation" has been added to the end of the third clause of claim 2. In view of the withdrawal of claim 1, the dependencies of claims 2, 5, 6, 9-11 have also been amended. Applicant submits that these amendments are supported by the specification as filed and that no new matter has been added to the claims.

Interview Summary

Applicants would like to thank Examiner Ewoldt for the courtesy extended during the telephonic interview on February 26, 2004. Applicants' attorney Robin A. Chadwick and Applicant's representative Elizabeth Jones participated in the interview with Examiner Ewoldt.

The pending claims and rejections under 35 U.S.C. § 112 were discussed during the interview. Applicants' representative provided background on the invention, explaining that the methods of the invention are not intended to kill the cells generated to express antigenic molecules on their surface. The Examiner suggested clarification of the claims in this regard. Use of the term "viable" before the word "cell" within the first clause of claim 2 was proposed. The Examiner had alleged that only one Example was provided in the specification and that this Example did not support the scope of the invention. Applicant's attorney and Applicant's representative explained that the specification and the Høgset Declaration submitted November 18, 2002 provide several Examples of the methods of the invention. These Examples and the Høgset Declaration were discussed and described in some detail by Applicant's attorney and Applicant's representative. Applicants' Attorney also asserted that Applicants need not describe in the specification what was known to one skilled in the art at the time of filing. The Examiner requested that the response to the October 2003 Office Action include a summary of these Examples and the Høgset Declaration as they relate to the rejections under 35 U.S.C. § 112. The Examiner indicated that if this was done, the Examiner would be favorably disposed toward allowance of this case.

This account is believed to be a complete and accurate summary of the interview as required by 37 C.F.R. § 1.133. If the Examiner believes that this summary is inaccurate or incomplete, Applicants respectfully request that the Examiner point out any deficiencies in his next communication so that Applicants can amend or supplement the interview summary.

35 U.S.C. § 112, First Paragraph, Enablement Rejection

The Examiner rejected claims 1-11 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement. The Examiner has alleged that the specification provides only a single, uncontrolled example relevant to the invention described in the claims.

Claim 2 is directed to a method of expressing an antigenic molecule on the surface of a viable cell, said method comprising: contacting said cell with said antigenic molecule and with a photosensitizing agent, wherein said molecule and said agent are each taken up into an intracellular membrane-restricted compartment of said cell; and irradiating said cell with light of a wavelength effective to activate the photosensitizing agent, such that the membrane of said intracellular compartment is disrupted, releasing said molecule into the cytosol of the cell, without killing the cell by irradiation, wherein, said released antigenic molecule, or a part thereof of sufficient size to generate an immune response, is subsequently presented on the surface of said cell by a class I MHC molecule.

Contrary to the Examiner's assertions, the full scope of the invention need not be taught by the specification alone. Instead, the test for enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation. *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 231 U.S.P.Q. 81, 94 (Fed. Cir. 1986), cert. denied, 480 U.S. 947 (1987) (emphasis added). The amount of guidance or direction needed to enable the invention is inversely related to the amount of knowledge in the state of the art as well as the predictability in the art. *In re Vaeck,* 947 F.2d 488, 20 U.S.P.Q.2d 1438, 1445 (Fed. Cir. 1991), citing *In re Fisher*, 166 U.S.P.Q. 18 (C.C.P.A. 1970).

Applicant has Provided Numerous Examples that Illustrate the Invention

The Examiner has stated that the specification provides just a single, uncontrolled example relevant to the claimed invention. Applicant submits that several examples are provided

in the specification and in the Høgset Declaration submitted November 18, 2002 that fully illustrate how to make and use the invention. In particular, Applicant directs the Examiner's attention to the following Examples that show cellular internalization and display of a variety of antigenic molecules.

Example 1 describes photochemical internalization of a labeled ras-peptide into fibroblast cells using $AlPcS_{2a}$ as photosensitizer and red light for photoactivation. The results of such an experiment are shown in Figure 2 of the application. As illustrated by Figure 2, when no light or no photosensitizer is used (top two panels of Figure 2), the peptide is taken up into intracellular compartments and the labeled peptide appears in discrete subcellular compartments. However, when both light and photosensitizer are used (bottom two panels of Figure 2), the labeled ras-peptide is found diffusely within the cytoplasm. Moreover, the cell remains intact -- evidence that the cells remain viable after internalization of the peptide and photoactivation of the photosensitizer.

Example 2 illustrates that MART-1 peptides are expressed on the cell surface after use of the methods of the invention. Example 2 further shows that those MART-1 expressing cells are killed upon addition of cytotoxic T cells that recognize the displayed MART-1 peptides. The Høgset Declaration provides further details on the experiments outlined in Example 2. As described in Example 2 and the Høgset Declaration, the MART-1 peptides were internalized into melanoma cells by the $AlPcS_{2a}$ photosensitizer using red light for photoactivation. Cytotoxic T cells (CTLs) were then added to test whether the CTLs recognize MART-1 peptides on the surface of the melanoma cells. The results of these experiments are shown in Figure 3 of the application, and in Figure 1 of the Høgset Declaration, which show that when no light is used little cytotoxicity is observed. However, when the MART-1 displaying cells are exposed to increased light, increased cytotoxicity is observed in a dose-responsive manner. Figure 1 of the Høgset Declaration also shows that when the assays are performed without addition of the MART-1 peptide, there is substantially no cytotoxicity. Hence, it is not the $AlPcS_{2a}$ photosensitizer that is cytotoxic. Instead, the cytotoxic T cells recognize the MART-1 peptide displayed on the surface of the cells and then kill those cells.

The results of experiments described in Example 2 and the Declaration, are summarized in the table below, which lists the components present in assays 1-5 and provides an indication of

which combinations of components gave rise to killing of the MART-1 antigen presenting cell by cytotoxic T cells (CTLs).

Assay	Photosensitizer	MART-1	Light	CTL	Cell Killing?
1	+	+	+	+	High cell killing
2	+	+	-	+	Little cell killing
3	+	+	+	-	Little or no cell killing
4	+	-	+	+	No cell killing
5	+	-	-	+	Little or no cell killing

These results give rise to three conclusions. First, cytotoxic T cells must be involved in cell destruction (rows 1 and 3). Second, light is necessary (rows 1 and 2). Third, the MART-1 peptide is required for CTL-mediated killing (rows 1 and 4). From these conclusions, it can be established, as Dr. Høgset declared, that photochemical treatment results in MART-1 internalization, processing and presentation on the surface of the cells in a form that is recognized by cytotoxic T cells, and that such presentation causes death of those cells by cytotoxic T cells (paragraph 4 of the Høgset Declaration). Prior to recognition and lysis by cytotoxic T cells, the cells were viable had not been killed by irradiation.

Example 3 describes photochemical internalization of horseradish peroxidase into NHIK 3025 carcinoma cells using $TPPS_{2a}$ as photosensitizer and exposure to blue light for photoactivation for various time periods. Total horseradish peroxidase activity for intact cells was compared to horseradish peroxidase activity in the cytosol, which was extracted by electropermeabilization and density centrifugation. Horseradish peroxidase activity was also measured in the cell corpses (centrifugation pellet) from which the cytosol had been extracted. As shown in Figure 4, the level of horseradish peroxidase in the cytosol (hollow circles) increases with increasing light exposure. The drop in total cellular horseradish peroxidase activity (filled circles) and in the cytosol (hollow circles) after 60 seconds likely indicates that the internalized horseradish peroxidase is beginning to be degraded prior to presentation on the

surface of the cell. Hence, photochemical internalization can give rise to internalized enzymes that are subsequently degraded by cellular machinery.

The Høgset Declaration also describes two additional experiments that further illustrate the effects of photochemical internalization of different types of molecules. In one of these experiments, a nucleic acid that encoded enhanced green fluorescent protein (EGFP) was complexed with polylysine, and this complex was internalized into HCT116 cells by use of the $AlPcS_{2a}$ photosensitizer with red light for photoactivation. As shown in Figure 2 of the Høgset Declaration, the majority of cells survived photochemical internalization of the polylysine-EGFP complex so long as the cells were exposed to less than about 5 minutes of photoillumination. Figure 2 also illustrates that the polylysine-EGFP complex was internalized because the number of EGFP-positive cells increased with increasing exposure to light (Høgset Figure 2). In order for EGFP-positive cells to be detected, the EGFP nucleic acid must have been transcribed and translated into EGFP protein. Hence, the only way that EGFP protein could be detected is if the photochemical internalization method actually internalized the EGFP nucleic acid into the cell without cellular injury sufficient to adversely affect the transcriptional and translational machinery of the cell.

The last experiment described in the Høgset Declaration illustrates that the combination of $AlPcS_{2a}$ photosensitizer and red light causes slower tumor cell growth but when this photosensitizer is used to internalize a cytotoxin (gelonin) into tumor cells, the volume of tumors in mice is substantially reduced. Balb/c (nu/nu) mice with subcutaneous WiDr tumors were injected intraperitoneally with $AlPcS_{2a}$. Forty two hours later, the cytotoxin gelonin was injected directly into the tumors of these mice and the mice were exposed to 135 J/cm^3 of light. Figure 3 of the Høgset Declaration shows the volume of tumors in mice treated in various ways. As illustrated, the tumors grew rapidly even after gelonin injection. Treatment of the mice with the $AlPcS_{2a}$ photosensitizer and light gave rise to slower tumor volume increases, but it is clear that the majority of the tumor cells remained viable (Høgset, Figure 3). Mice that received $AlPcS_{2a}$ as well as light and gelonin had substantially smaller tumor volumes for up to 60 days after treatment. These results indicate that when cytotoxic molecules are internalized using photochemical internalization methods, those cytotoxic molecules can kill the cells into which they have been internalized.

As described in more detail below, when internalized by photochemical internalization, non-toxic peptides and polypeptides do not cause such cell killing. Instead, those non-toxic peptides and polypeptides can be fragmented and displayed via the MHC pathway on the surface of the cells into which they have been internalized.

Therefore, contrary to the Examiner's allegations, the specification and the Høgset Declaration provide numerous examples of different polypeptides and peptides that can be internalized into various cell types using different types of photosensitizers and different light sources and then presented on the surface of those cells for interaction with immune effector cells.

Moreover, Applicant submits that one of skill in the art need not teach what is available in the art. As described in more detail below, the art provides numerous examples of photochemical internalization of numerous molecules into numerous cell types using a variety of photosensitizers and light conditions. Hence, one of skill in the art can readily practice the invention using the teachings of the specification and the art.

Cell types

WO96/07432 (D1) further illustrates that photochemical internalization occurs in many cell types. For example, the examples provided in WO96/07432 describe transport of proteins into NHIK 3025, V79, H146, COS-7 and OHS cells. The last paragraph on page 11 of WO96/07432 indicates that a variety of different photosensitizers and doses can be used to introduce different molecules into the cytosol of cells. Thus, the method of the present application would be expected to work on a variety of cell types.

Moreover, Selbo *et al.* (*Int J Cancer*, *87*, 853 (2000); copy previously provided) illustrates internalization of MOC 31-gelonin in which the monoclonal antibody MOC31 is conjugated to the cytotoxin gelonin. Selbo *et al.* (2000) also demonstrates that such internalization is effective in five different cell types (NCI-H146, KM20L2, WiDr, T-47D, and THX). Successful internalization is apparent from the figures, which show that the use of gelonin alone is ineffective in reducing protein synthesis and that this cytotoxin only has its cytotoxic effect upon transfer to the cytosol, which is only achieved using photochemical internalization. Selbo *et al.* (2000) also documents the efficacy of two different photosensitizers

from internalization, namely, $TPPS_{2a}$ and $AlPcS_{2a}$. Thus, Selbo *et al.* (2000) is a further example of internalization of a protein using the photochemical internalization method.

Photosensitizers

A variety of photosensitizers have been shown to be useful for photochemical internalization. For example, WO96/07432 describes the use of $TPPS_{2a}$, $AlPcS_{2a}$, and $TPPS_4$. Furthermore Selbo *et al.* (*Photochemistry and Photobiology, 74*, 303 2001; copy previously provided), shows that 5-aminolevulinic acid (5-ALA) can be used for photochemical internalization. For example, Figure 3 of Selbo *et al.* (2001) showed that gelonin alone does not affect cell viability (solid squares) and that only 5-ALA had an effect on cell viability as illumination time increased (solid circles). In contrast, when 5-ALA and gelonin are used in combination (open triangles), cell viability is significantly compromised, indicating that the gelonin was internalized into the cells. Thus, a variety of different photosensitizers and procedures for using these photosensitizers are available. For the present methods, photochemical internalization is performed on cells with MHC Class I molecules and presentation will therefore occur when various photosensitizers are used.

Antigenic molecules

It has been demonstrated that photochemical internalization works well with a variety of different classes of substances ranging from low molecular weight drugs, a ras-derived peptide, a MART-1 peptide, horseradish peroxidase, gelonin and various other proteins. For example, in addition to the teachings of the specification, the WO96/07432 publication illustrates internalization of the proteins gelonin, agrostin, and saporin. Thus, a variety of molecules, including particular proteins, can be internalized. The present application shows that following internalization, appropriate presentation via the MHC Class I machinery occurs.

Viable Cells

The Examiner has indicated that the specification is limited to a teaching of killing melanoma cells by addition of a highly toxic substance ($AlPcS_{2a}$). Office Action at page 2-3 (Oct. 24, 2003). The Examiner has further cited to a statement in a Declaration by Høgset

(submitted November 18, 2002) that describes upon which factors cell death is principally dependent.

Applicant submits that, contrary to the Examiner's allegations, the invention is not directed to killing cells. Instead the invention is directed to methods of expressing an antigenic molecule on the surface of a viable cell. In Example 2, cell death is caused by cytotoxic T cells, which are used simply as a way of confirming the existence of viable MART-1 presenting cells prepared by the present methods. Discussion of cell death in the Høgset Declaration was directed to avoiding substantial cell death so that a viable cell could be generated that expressed the antigenic molecule of interest on its surface.

Applicants submit that the confusion about cell killing may arise from confusion of the present methods with photodynamic therapeutic methods. Before the advent of the present invention, photodynamic therapy was used, typically for killing certain undesirable cell types. During photodynamic therapy, photosensitizers were administered to, and taken up by, cells. The cells containing the photosensitizers were then illuminated, causing activation of the photosensitizers, which in turn either directly or indirectly generated toxic species. When such toxic species were present in high enough levels, the cells would die, thereby eliminating or killing the undesirable cells. Hence, photodynamic therapy has been used to achieve targeted cell death. Such methods are currently used successfully and routinely for *in vivo* treatment of tumors. However, the invention is not directed to methods involving such photodynamic therapy and cell death is not the goal of the present methods.

After photodynamic therapy became known, the inventors found that using low levels of photosensitizers or shorter illumination times reduced cell death and, instead, led to disruption of internal cellular membranes and release into the cytosol of molecules trapped within intracellular compartments. This procedure came to be known as photochemical internalization. Photochemical internalization procedures permitted transfer of desirable molecules into the cell cytosol. For example, photochemical internalization has been used for internalizing DNA into cells for gene therapeutic purposes. Some workers also used photochemical internalization for delivery of toxic proteins into tumor cells when photosensitizer activation itself had not resulted in cell death, for example, within the centers of tumors. This type of internalization has been shown to occur *in vivo*, for example, as shown in Figure 3 of the Høgset Declaration submitted November 18, 2002.

In the present invention, photochemical internalization is coupled to antigen presentation via the major histocompatibility complex (MHC) for presentation of peptides derived from non-toxic proteins while maintaining the viability of the cells. According to the invention, molecules that are internalized into the cell by photosensitizer activation can surprisingly be processed by the MHC presentation system and presented on the cell surface. One of the major problems with generating immune responses by methods available prior to the invention was the inability to introduce sufficient amounts of antigen into cells. Without sufficient internalization of the selected antigen, the cells could not display sufficient antigen on their surfaces. The present invention solves this problem by provided a much needed mechanism for internalizing and therefore presenting antigens on cell surfaces.

Internalization and presentation by the methods of the invention allow cellular display of peptides that previously could not be presented on cells, or were presented in only very low levels because of low uptake into the cell. The method thus facilitates generation of immune responses to peptides that might otherwise not be presented properly for eliciting such an immune response.

The Examiner has also suggested that optimizing internalization rather than cell death is unpredictable and that the skilled person is not provided with details of all the factors that should be taken into account and how they should be varied. Applicant respectfully disagrees. As described above, photochemical internalization is a technique which is described in detail in the specification and in WO96/07432 (D1). For example, WO96/07432 (D1) shows the effect of time of irradiation on internalization, the use of different agents and the use of different concentrations of the molecule to be internalized (a toxin molecule as shown in WO96/07432). WO96/07432 (D1) teaches that the viability of the cell is retained by modifying the conditions used for internalization accordingly to maximize internalization, but minimize cell death (see the passage bridging pages 2 and 3 in D1). See also, for example, Example 2 in WO96/07432 (D1) which discusses how to vary the level of survival by appropriate selection of the photosensitizer and the light exposure, and Example 3 which illustrates that light doses control the amount of molecules released into the cytosol. See also Examples 4 and 5, which illustrate that viability of the cells can be largely unaffected.

Thus, Applicant respectfully submits that the optimization necessary to obtain the appropriate light, cell, antigenic molecule and photosensitizer conditions is taught in the

specification and the art. Furthermore, the optimization is not unpredictable. Higher intensity light or longer irradiation times will lead to more cell damage and hence cell death. Higher photosensitizer doses will lead to higher cell damage and hence cell death. The skilled person needs simply to either increase the levels of these factors until maximum internalization is achieved before significant cell deaths occurs, or if cell death is occurring, reduce the levels of these factors until that cell death reduces. In all the experiments conducted thus far, as the parameters are initially increased, internalization increases. At some point, however, as the parameters are increased further, cell death increases. Thus, a graph may be plotted showing internalization versus cell death as the parameters are increased. This is what is shown in the Høgset Declaration previously submitted by the Applicant in Figure 2 which shows that transfer of molecules can be achieved with a minimal loss of survival. The skilled person can select an appropriate set of parameters at which maximal internalization has occurred with minimal cell death. Furthermore, this optimization involves routine screening techniques, and routine screening techniques do not constitute undue experimentation. *In re Wands*, 8 U.S.P.Q.2d 1400, 1406-1407 (Fed. Cir. 1988).

Therefore, the specification illustrates that photochemical internalization gives rise to little or no cell death as described below. Moreover, the specification provides examples that illustrate cellular display of photochemically internalized antigens as described above.

Applicant therefore asserts that the specification fully enables one skilled in the art to use the method of the present invention. The first paragraph of 35 U.S.C. §112 requires no more than a disclosure coupled with information known in the art enables one skilled in the art to carry out the invention, and this requirement has been met. It is respectfully submitted that the pending claims are in compliance with 35 U.S.C. §112, first paragraph. Therefore, Applicant requests that the Examiner withdraw the 35 U.S.C. §112, first paragraph enablement rejection of the claims.

35 U.S.C. § 112, First Paragraph, Written Description Rejection

The Examiner has also rejected claims 1-11 under 35 U.S.C. § 112, first paragraph, as allegedly lacking written description. Applicant submits that, as described above, in the present invention, photochemical internalization is coupled to antigen presentation via the major histocompatibility complex (MHC). Hence, available teachings on photochemical

internalization provided, for example, in WO96/07432 (D1), can be used in conjunction with the teachings of the application to practice the invention. Thus, it is submitted that the, written description requirement has been met and Applicant respectfully requests withdrawal of the written description rejection under 35 U.S.C. § 112, first paragraph.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is respectfully requested. The Examiner is invited to telephone Applicant's attorney (516-795-6820) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,
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CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on this 23rd day of March, 2004.

PATRICIA A. HULTMAN

Name

Signature